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19. ABSTRACT (Continue on reverse if necessary and identify by block number)
The aims of these experiments are to use intact corneas of rabbits exposed in vivo to high energy pulsed millimeter waves to study the development of corneal damage. Such experiments are expected (1) to establish conditions for corneal damage and (2) to elucidate the mechanisms by which the damage occurs. The lowest level to produce damage to date is 0.03 W/cm^2 pulsed irradiation.

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SUMMARY

Techniques were developed for irradiation of rabbit eyes with 35 GHz millimeter waves to determine cellular damage mechanisms and thresholds. The irradiation was performed using anesthetized 2.5 kg New Zealand white rabbits exposed to 35 GHz pulsed and continuous wave (CW) irradiation delivered by a special focusing antenna to a spot approximately 1.3 cm diameter in which the rabbit eye was placed. The peak specific absorption rate (SAR) (1.4 mW/g per 1 mW/cm^2 of incident power) was determined with the aid of a thermographic camera which gave a map of the local corneal surface temperatures; the SAR assumed the values of thermal conductivity and specific heat for water since the cornea contains so much water. The damage observed has been divided into four categories which are progressively increased, by SEM and TEM evaluation. The unirradiated left cornea appears to serve as an appropriate control. This damage appears to increase (1) as the irradiation is modulated by pulsing at the same average power. (2) Damage increases as the same total energy is delivered over a short period (15 min vs 0.2 sec), perhaps as a result of higher temperature values attained. (3) the lowest dose rate at which damage has been detected in preliminary experiments is 0.03 W/cm^2 (SAR 33 mW/g) for pulsed irradiation for 15 min exposure. These experiments should be continued to permit further data to be gathered, since the threshold value implicit in the results above is rather close to the safety threshold, when the safety factor of 10 is taken into account.

Keyword: Non ionizing radiation; Irradiation; eff... (A W)

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FOREWORD

A. List of professional Personnel Employed on This Project

Principal Investigator	Dr. John R. Trevithick, Ph.D.
Research Consultant	Dr. Margaret O. Creighton, Ph.D.
	Dr. Maurice Hirst, Ph.D.
	Dr. A.P. Cullen, Ph.D..
	Dr. S.E. Sanford,D.V.M., Dept. of Vet. Pathol.
Research Associate	Dr. Madhu Sanwal, Ph.D.
Technician	Mr. T. Dzialoszynski

B. Animal Care

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and use of Laboratory Animals" prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 86-23, Revised 1985).

TABLE OF CONTENTS

INTRODUCTION

Although millimeter wave radars are now strategically important, only one study of the effect of millimeter waves on the cornea has been reported (Rosenthal et al., 1975). This study did not use the pulsed mode of millimeter waves which is commonly in use in such radars. Because we have discovered apparent differences between similar doses of pulsed and CW microwaves (Stewart-DeHaan et al., 1980) in preliminary experiments and because we have succeeded in separating the effect of heating from the effects due to the electromagnetic field, for microwaves, we wished to devise a similar system for irradiation of the cornea in vitro which would offer similar advantages for the study of millimeter wave damage to the cornea.

The first step in these experiments was to devise appropriate media and conditions for the tissue culture of corneas, to be used for the experiments investigating their exposure to millimeter waves. The second step, also described in our Annual Report, June 1981 under DAMD17-80-G-9480, was to incubate the cultured corneas at different elevated temperatures in order to investigate the effect of incubation at elevated temperature on the cornea. The third stage, reported in our Annual Report, June 1982 under DAMD17-80-G-9480 and DAMD17-82-C-2018, was to test the effect of exposing incubated corneas to non-ionizing radiation, for which we selected ultraviolet as convenient and accessible in our laboratory, since the appropriate millimeter wave irradiation apparatus was not yet operating. In 1983 the millimeter wave irradiation apparatus was functioning, permitting us to perform preliminary experiments on rabbits in vivo which were reported in the annual report (June 1983) for DAMD17-82-C-2018.

In the interim period Kues and his collaborators have reported deleterious effects of 2.45 GHz microwaves (Kues et al., 1985) in which for pulsed irradiation, a lower exposure level was necessary to induce equivalent damage when compared to CW irradiation. The incident irradiation levels these investigators used were at 10 mW/cm^2 or more, with a total power input of approximately 2.9 W with 50-150 mW reflected for the 10 mW/cm^2 condition, but the SAR was not determined. A latency period of approximately 1 day was required for the effects to apear, and the effects did not appear to be thermally caused, since only minimal temperature elevations occurred.

The experiments reported here use several novel concepts to permit further information to be obtained regarding MM wave effects.

1. A focusing antenna permits a pencil beam of millimeter waves to be delivered to the target.
2. Since target and antenna are physically separated, air cooling is possible.
3. The surface temperature at any point on the target may be monitored continuously and recorded at intervals as small as 0.1 sec using the AGA thermovision computerized camera.
4. A computerized pachometry apparatus permits accurate records of corneal thickness to be obtained before and after irradiation at various times.
5. Photographic recording of corneal appearance by slit-lamp biomicroscopy, and retro-illumination photography of the cornea is combined with real-time video recording of the corneal appearance during irradiation by MM waves.

6. Development of a defined protocol for division of the cornea into areas to be used for SEM, LM, and TEM, and a defined procedure for examination of the divided areas by SEM and LM (and TEM as appropriate).

7. Quantitation of the damage using a Zeiss videoplan to obtain a statistically valid measure of the millimeter-wave-induced damage.

MATERIALS AND METHODS

Outline of Experimental Details

Animal handling

New Zealand white rabbits were used in this study, housed in part at the University of Western Ontario Animal Quarters and at the Walter Reed Army Institute of Research. For in vivo irradiation at the WRAIR, rabbits were anesthetized using ketamine – Rompun (xylazine) prior to and during irradiation of their corneas (see details below under year 1). Corneal temperature was monitored using a thermographic camera and when desired maintained by a current of moist air across the corneal surface. Irradiation was performed as described below. Following irradiation in vivo, after an appropriate period of latency, rabbits were reanesthetized (see details) for slit lamp and pachometry examination. Analgesia was provided as necessary to alleviate discomfort. At the end of the experiment, rabbits were killed by euthanasia with pentobarbitol, 230 mg/kg. The eyes were removed and the corneas dissected out, being careful not to damage the epithelium and endothelium – this was checked by scrutinizing each under a dissecting microscope.

Anesthesia

Rabbits were anesthetized by 30–50 mg/kg ketamine with Rompun (5–10 mg/Kg) injected intramuscularly. This does not have the side effect of lowering animal body temperature.

Materials and methods

The anesthesia conditions were tested at the University of Western Ontario. Pulsed millimeter wave exposures took place at the WRAIR millimeter wave exposure chamber in Building 40. All exposures were at a frequency of 35 GHz. The exposure conditions used (Table 4) explored duration, peak power and total dose required for perceptible damage, as well as latency, in a preliminary way.

In vivo irradiation

New Zealand white male rabbits, average weight 2.5 kg were irradiated using a special focusing antenna in a special rabbit carrier constructed of plexiglass and nylon parts which maintained their heads in a fixed position (Fig. 1) during the 15 min period of irradiation. The carrier was fastened to a large block of styrofoam by strong plastic lacing so that the optical axis of the rabbit's eye approximately coincided with the axis of the focussing antenna emitting the millimeter waves. The distance from the cornea to the front of the antenna was measured. For SAR determination the rabbit's corneal surface temperature was measured by recording the temperature reading of a thermographic camera.

RESULTS AND DISCUSSION

These results represent the first irradiation at 35 GHz which did not involve a contact applicator, and thus do not suffer from possible heating artefacts associated with contact applications used in previous experiments. The cornea would experience similar effects when exposed to an outdoor millimeter wave radar transmitter source (ie. suface absorption only). In order that the damage which occurs can be documented, it was necessary to devise methods for the following:

1. Slit lamp biomicroscopic examination of the rabbits and photographic recording of the results.
2. Anesthesia.
3. Measurement of temperature of cornea using thermographic camera and calculation of SAR (specific absorption rate) during irradiation by 35 GHz waves.
4. Cooling of corneas by air cooling.
5. Computerized recording by pachometry of thickness of cornea.
6. Reproducible dissection and fixation of corneas.
7. Division of cornea into sections for examination by SEM and TEM.
8. Reproducible examination of samples.
9. Classification of types of damage.
10. Quantitation of damage using Zeiss videoplan.

1. Slit Lamp Biomicroscopy

We have used this technique to observe changes between the appearance, Zeiss-slit lamp biomicroscope of the cornea, before and after irradiation, by retro-illumination, and slit-lamp illumination, both coupled with photography and pachometry using a computerized apparatus to quantitate changes in corneal thickness.

2. Anesthesia

Experiments with anesthesia of the rabbits revealed that the corneal reflex was abolished reproducibly for the period between 10 and 25 min. from the initial injection using ketamine (35 mg/kg) and xylazine (5 mg/kg). The reflex returned slowly towards normal, which was attained after 60 min. from the initial injection.

3. Measurement of Corneal Temperature with Thermographic Camera

Figure 2 illustrates the rabbit head in holder by photography (a), thermography (b) and thermographic profile (c). The temperature of the cornea was measured using an AGA thermovision 780 system equipped with a BMC computer analysis system programmed using the disco 2.0.1 system (Gesotec, Federal Republic of Germany), for image recording and analysis. The range of corneal temperatures observed was 19.5–32°C. A relatively flat oblong plateau at the maximum temperature (32°C) occupied the central area of the cornea rising steeply from the lower temperature at the bottom and the top of the eye.

Phantom SAR studies for millimeter wave irradiation

(a) spot size: Thermographic images of the temperature distribution immediately before and after millimeter wave irradiation of the corneal phantom were analyzed at a round spot within the center of the phantom. Here the most significant surface temperature elevation occurred (Fig.   ). Computer image-processing was used to analyse the termographi subtraction image (including vertical and horizontal linear-scan profiles) that were obtained following subtraction of final initial temperatures found on exposure to the millimeter wave irradiation. The vertical and horizontal scan-line profiles that passed through the center of the spot indicated smooth curves with no apparent hot spots or sharp increases in temperature within the spot (Fig. 3b,c). The spot size of the region that experienced a measurable temperature rise during irradiation was defined by using the width of the area where the temperature risewas one half of the maximum value of the peak temperature rise. The absolute distance across the corneal surface were estimated by calibrating the termographic camera's spatial display with a heated metal reference object. This rectangular object was imaged at the same camera-to-subject distance as the camer-to-cornea distance that was used during the millimeter wave SAR experiments. Using this approach, the horizontal widths of the millimeter-wave heates spot on the phantom were found to be 0.7 cm (Fig. 3b) when the phantom was surface positioned at the focal point of the transmitting antenna. When the phantom surface was placed at distances of 2.5 cm (Fig. 3d) and 5 cm from the antenna's focal point, farther along the axis of propagation the horizontal widths of the heated spot were 1.25 cm (Fig. 4d) and 2.3 cm (not shown) respectively. The vertical heights of the heated spots were 1.09 (Fig. 4e) at the antenna focal point, 1.35 cm (not shown) for an antenna-to-phantom distance that was 2.5 cm father from the focal point, and 2.8 cm when the separation distance was increased to a distance that was 5 cm from the focal point. A similar sized spot was estimated for the rabbit eye (Fig. 4)

Peak SAR values for cornea and phantom

The peak SAR was estimated using the maximum temperature elevation at the peak of the temperature plot. The calculation of the SAR utilized the following approximations for the value of the specific heat of the irradiated objects. Since the tissue of the cornea and the cucumber have a very high water content, the values for the specific heat and mass density were used. Using this approximation, the spatial peak SAR values calculated for the phantom, (obtained for an average radiated power of 4W) were 1.1 W/kg per Watt of average transmitted power, or 1.46 +/-0.3 mW/g per mW/sq cm of radiated power. For the rabbits, an average radiated power of 0.38 W was used to obtain an SAR value 1.1 W/kg per Watt of average transmitted power, or 1.43 + 0.34 mw/g per mW/sq cm of radiated power. These results indicate that the SAR induced in the cornea of the rabbit and in the phantom (cucumber) are nominally identical. For this reason, it appears that use of a cucumber as phantom was a useful and practical alternative to the sacrifice of rabbits. This is especially true for the preliminary experiments involving the determination of the optimum location of the subject being irradiated, and for the determination of corneal SAR (magnitude and spatial distribution). Also, the se of the phantom offered advantages of (1) not requiring animals subjected to anesthesia and (2) permitting effects of higher average powers (4W) to be tested without air cooling. This, in turn, enabled higher signal to

noise ratios to be obtained during the acquisition to thermographic data.

4. Corneal Cooling by Air Flow

Air flow from a Fisher vacuum/pressure medical pump through a gas-washing bottle containing water at $50^\circ C$ equilibrated the air with water vapour, so that at the temperature of released air ($20^\circ C$ approximately) blowing on the rabbit eye, the relative humidity of the air was 80% or greater. Several air flow rates were used, resulting in differential cooling. At a particular flow rate (10 psi pressure at pump corresponding to 16.9 l/min air flow), the corneal peak temperature elevation was a linear function of the incident power. When the air flow rate was increased with no heating, the temperature decreased linearly with increased flow rate (Fig. 5).

Influence of power applied and air flow on corneal heating

Millimeter wave heating by continuous wave irradiation was studied in the absence of air flow. Increasing the power applied to an air flow rate of 17 l/min resulted in an increase in temperature of the hottest point on the corneal surface, which was a linear function of the applied power (Fig. 6), within the range of power up to 2 W average power. The addition of air cooling resulted in a lower increase in temperature for a given level of radiated power. However, the increase in temperature was still a linear function of the power used for heating the cornea. This seemed to agree with the earlier findings from the corneal cooling experiments that were performed in the absence of millimeter wave irradiation. As the air flow rate was increased for a constant radiated power level, the amount of temperature rise decreased with higher air flow rates resulting in the observation that the temperature elevation with no air flow at 0.75 W/sq cm was identical to that with 34 l/min air flow at 2.25 W/sq cm. thus 34 l/min apparently provides cooling equivalent to 1.5 W/sq cm.

5. Computerized Pachometry Measurement of Corneal Thickness

Dr. A. Cullen has arranged for computerized operation of a Zeiss pachometer he has modified electronically, when activated by a foot switch, to record the reading and average 7 or 8 successive readings to give a result for each of average +SE. It was possible to estimate differences between corneal thickness, before and after exposure, to an accuracy of $1 \mu m$. In later experiments no increase between initial and final thickness occurred as a function of microwave irradiation. For this reason this technique will only be used for experiments involving latency which afford an opportunity for corneal swelling to occur.

6. Fixation of Corneas

The corneal epithelium was pre-fixed in situ immediately following euthanasia of the rabbit, with two drops of Karnovsky's fixative. After a 2-3 min. pre-fixation period, the cornea was cut off with a rim of sclera. After making a nick to identify the nasal area, it and the other portions of the eye were fixed with gentle agitation in Karnovsky's fixative at $4^\circ C$. The tissues were fixed for $4^\circ C$ for 24-48 hr then transferred to 0.1 M sodium cacodylate buffer, pH 7.2.

7. Division of Corneas for Examination by SEM and TEM

Figure 7 shows the diagram of dissection of the corneas into segments for examination by Scanning Electron Microscopy (SEM), light microscopy (LM) and Transmission Electron Microscopy (TEM) is attached. The center strips are removed (see below) and opposing segments (1 & 3 or 2 & 4) are placed together in a small vial containing fixative or cacodylate buffer. The sections 1 & 3 will be used to view epithelial surfaces, the sections 2 & 4 to endothelial surfaces. The sections are processed in the usual manner for SEM (dehydration through alcohol series and critical point drying, then afterwards attached to aluminum stubs with either silver daube paint or nickel paint and spattered with gold palladium). The 2 mm wide strip taken at the equatorial zone was put away as sample C. The nasal area was marked by a diagonal cut. After both anterior and poasterior halves of the cornea were bissected, 2 mm strips were taken from the middle and labelled A and B. As before, a diagonal cut was made at the central area. A, B, C are processed of LM and TEM. They are post fixed in 1% osmic acid for 2 hours, washed, dehydrated and prepared for embedding in Epon or Spurr resin. After the final stage of resin infiltration, the long strips of cornea are placed on a piece of dental wax and cut in 1.5 mm blocks by a sharp blade. The cut pieces of tissue are embedded in numbered flat embedding models; #1 being the diagonally cut end. This enables us to locate the exact position of any block being studied. Generally, semithin sections are cut from alternate numbered blocks, stained with toluidine blue and viewed under LM for general morphology, and pertinent areas photographed. Some representative areas are selected and ultrathin sections are cut to be studied under TEM after staining with uracyl acetate and lead citrate.

8. Reproducible Examination of Samples

a) **By SEM**: The corneas, dissected as illustrated in Fig. 7, on the stub were viewed systematically three viewing lines were established, at 500 µm, 1000 µm, and 2000 µm from the sclera (see Fig. 7 cutting diagram) – both epithelial and endotheial surfaces. Initially each specimen was scanned completely along these lines and 3 pictures taken at three randomly choosen spots on this line at 100X, 500X, 1000X, 5000X as indicated by Dr. Michael Doughty. At 100 X the percent area of % of lesions could be assessed; at 500X the light cells vs the dark cells could be compared; at 1000X the state of the cell border could be noted. However, such extensive photography was very expensive. It was therefore decided to take one picture at each of the 3 viewing lines at 500X for epithelial and 1000X for endothelial, after a visual inspection and conscious decision to photograph one specific area, as typical of the cell's condition at that view line. The pictures thus obtained were evaluated using a computerized Zeiss videoplan which gives individual cell areas, mean cell area and standard deviation, as well as cell perimeters. Using this method, areas of normal and abnormal cells for each rabbit cornea could be found and corneal comparisons made.

b) **LM and TEM Thick and Thin Sections**

9. Types of Damage Found

a) **Irradiation conditions**: It will be noted that most trials had moist air

flow over the cornea and that the rabbit was sacrificed immediately. However, Table 1B, immediately sacrificed animals, which were the last rabbits done in June 1986 (ie: #48-52) had no air cooling since it was found in experiments analysed by this time that the moist airflow was damaging the cornea. Possibly the various conditions on Table 1B should be done to compare no airflow with those of Table 1A in which air cooling was used.

Table 2 gives the same information in list form. It should be noted that rabbits 1-6 are composite experiments being given different sequential doses over 15 mins. Some information as to their final handling was lacking in the original notes. They could not easily be put on Tables 1A or 1B so are listed solely on Table 2.

b) <u>SEM</u>: (i) Visual library: During the assessment of the rabbit corneas examples of various degrees of damage were photographed and a simple system of nomenclature given to indicate degree of damage. N = Normal, Type I (a) – single cells missing, appear lifted whole from surface leaving "cooked" or coagulated area where cell was (Fig. 8); (b) numerous cells missing – like to Type Ia (Fig. 8a); (c) areas of single cells running together, like to Type Ia (Fig. 8b). Type II, similar to Type I but area more extensive a-c (Fig. 8c). Type III, whole area of 500X photo is damaged (Fig. 8d). As yet the assessment of the level of damage is visual; however, it is hoped after numerous corneal tests a more elaborate quantitative method of designating damage will be evolved, to supplement the videoplan data below.

(ii) Videoplan assessment of percent damage as a function or area: This shows the percentage of epithelial cells damaged as compared to the total number of cells visualized at a constant magnification of 500X at the various predtermined lines (500 μm, 1000 μm, 2000 μm from the sclera towards the center of the cornea) on the corneal surface. It would appear that except for certain exceptions (rabbit #3, rabbit #49) the unexposed left cornea acted as a good control to the experiment. Visual observation of each cornea showed that damage tended to be in lines on the surface, as if the damaging force skipped over it like a stone leaving a track of damage behind. Although 450W did do damage which was extensive where it was found, perhaps the short time of exposure, 0.2 sec, may account for untouched areas. Even 0.03W over a long – 15 mins. – resulted in some damage. More time course studies should be done.

(iii) Comparison of Pu-CW irradiation and pulse-power and time combinations: The accompanying table shows the samples from comparable exposure conditions at similar total input energy.

c) <u>Light microscopy of thick plastic sections</u>: Light microscopy revealed several types of damage, presented in order of increasing amount: Type L1 (Fig. 9a) outer epithelial cells lifting off as shown by arrow; Type L2 (Fig. 9b) in addition to lifting cells, the outer epithelial cells have groups of darkly strained cells which are apparently heavily damaged and thus degenerating; Type L3 larger areas of cornea (Fig. 9c) with more severe cell loss including complete loss of epithelial cells in some areas with exposure of the stromal surface; Type L4, light staining in the upper layers is consistent with cell death, while lower epithelial cells are more darkly staining indicating severe damage.

CONCLUSIONS

This study has been an introductory survey of corneal effects of pulsed (Pu), 35 GHz millimeter waves and a comparison with damage by continuous wave (CW) at similar average powers and defined specific absorption rate (SAR) values. Based on these results it is recommended that animal handling be minimized and air flow be 5 psi or lower. Use of the left cornea (unirradiated) as control seems to be appropriate providing no damage occurs during animal handling.

Several findings, although preliminary and requiring further confirmation, should be noted as potentially important for safety standard revisions which may eventually be contemplated by regulatory authorities. (1) the damage observed using pulsed irradiation appears to be greater than for CW at similar average powers. (2) The lowest average power investigated produced at significant damage for pulsed irradiation. This was at 0.03 W incident power (spatial peak SAR 33 mW/g temporal). This should be repeated to comfirm these results. This value expressed as power density over a 1.3 cm diameter spot is approximately $0.3/1.327$ cm^2 = 0.0226 mW/cm^2 which is only a factor of 4 above the safety limits suggested for whole body irradiation at this wavelength and also a factor of 4 above the local SAR ("partial body exposure") safety limits. (3) The finding that damage appeared more extensive for pulsed (modulated) than CW irradiation at similar average powers suggested that our previous findings of more damage from pulsed than CW irradiation also is true at 35 GHz as well as at 0.918 GHz. More work is required to accurately quantitate the factor by which the damage is increased because of modulation of the iradiation, and to explore the effect of pulse parameters, exposure duration, average power, temperature elevation and air cooling on the effect observewd. Further work is needed to investigate the effect of lower powers on corneal damage, to determine whether significant damage occurs at lower power levels, and whether the threshold is different for pulsed as compared to CW irradiation.

Comparison of total dose in Joules between our work and that of Kues et al. (1985) reveals a striking similarity between conditions he found damaging for pulsed microwaves (150 J/g) and our exposures 54 J/g at the minimum level at which detectable damage was found.

Overall Conclusions from These Experiments

1. A low airflow of 5 psi (group C exp) would seem to be most ideal - better than no air or air flow of a greater psi.

2. The higher the wattage the greater the damage to the cornea.

3. At higher wattages the damage area seems confirmed to the central region at which the millimeter waves are targetted.

4. Millimeter waves modulated by pulsing seem to be more damaging than CW at similar average powers.

5. Handling of the animals should be kept at a minimum as damage results easily.

6. The left cornea can be used as a control but there may be background damage due to handling which must be taken into account.

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Table 1A

Sacrificed Immediately: Pulsed

A — SACRIFICE AT 72 HOURS
B — SACRIFICE AT 48 HOURS
C — SACRIFICE AT 24 HOURS
D — SACRIFICE IMMEDIATELY

TIME	WATTS 0	.03	.1	.5	1	1.5	2	3	5	10	100	200	300	450
0														
.1 sec														
.2 "														
.9 "														
20 "														
1 min														
1.5 "														
2.5 "														
5 "														
7.5 "														
10 "														
15 "														

Table 1A

Sacrificed at 24 hr: Pulsed

A SACRIFICE AT 72 HOURS

B SACRIFICE AT 48 HOURS

C SACRIFICE AT 24 HOURS

D SACRIFICE IMMEDIATELY

WATTS

0	.03	.1	.5	1	1.5	2	3	5	10	100	200	300	450

TIME

0

.1 sec

.2 "

.9 "

20 "

1 min

1.5 "

2.5 "

5 "

7.5 "

10 "

15 "

Table 1A

Sacrificed at 48 hr: Pulsed

A	SACRIFICE AT 72 HOURS
B	SACRIFICE AT 48 HOURS
C	SACRIFICE AT 24 HOURS
D	SACRIFICE IMMEDIATELY

WATTS	0	.03	.1	.5	1	1.5	2	3	5	10	100	200	300	450

TIME

0

.1 sec

.2 "

.9 "

20 "

1 min

1.5 "

2.5 "

5 "

7.5 "

10 "

15 "
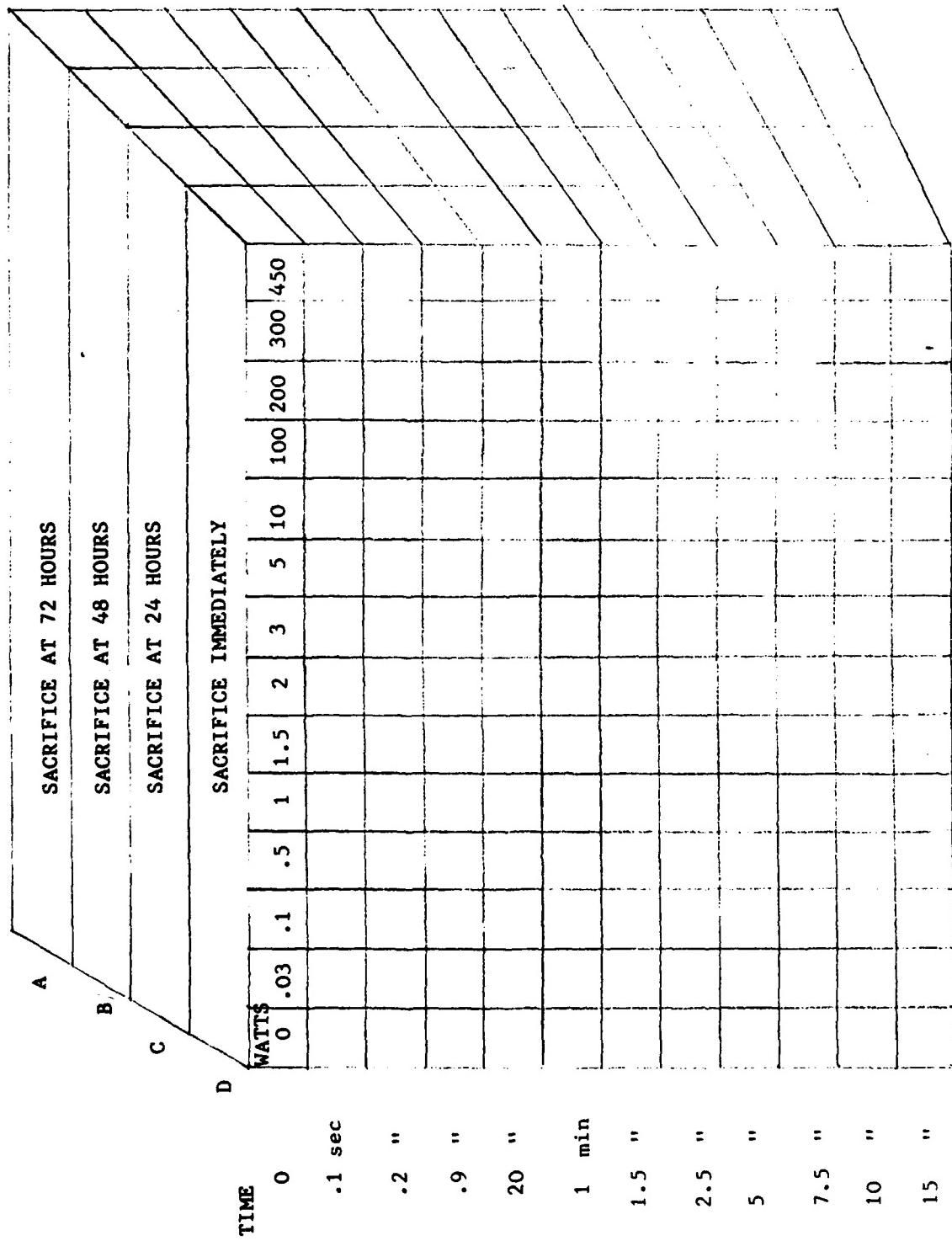
Table 1A

Sacrificed at 72 hr: Pulsed

A — SACRIFICE AT 72 HOURS

B — SACRIFICE AT 48 HOURS

C — SACRIFICE AT 24 HOURS

D — SACRIFICE IMMEDIATELY

WATTS: 0 | .03 | .1 | .5 | 1 | 1.5 | 2 | 3 | 5 | 10 | 100 | 200 | 300 | 450

TIME:
0
.1 sec
.2 "
.9 "
20 "
1 min
1.5 "
2.5 "
5 "
7.5 "
10 "
15 "

Table 1B

Sacrificed Immediately: CW

A — SACRIFICE AT 72 HOURS

B — SACRIFICE AT 48 HOURS

C — SACRIFICE AT 24 HOURS

D — SACRIFICE IMMEDIATELY

WATTS: 0 | .03 | .1 | .5 | 1 | 1.5 | 2 | 3 | 5 | 10 | 100 | 200 | 300 | 450

TIME:
0
.1 sec
.2 "
.9 "
20 "
1 min
1.5 "
2.5 "
5 "
7.5 "
10 "
15 "
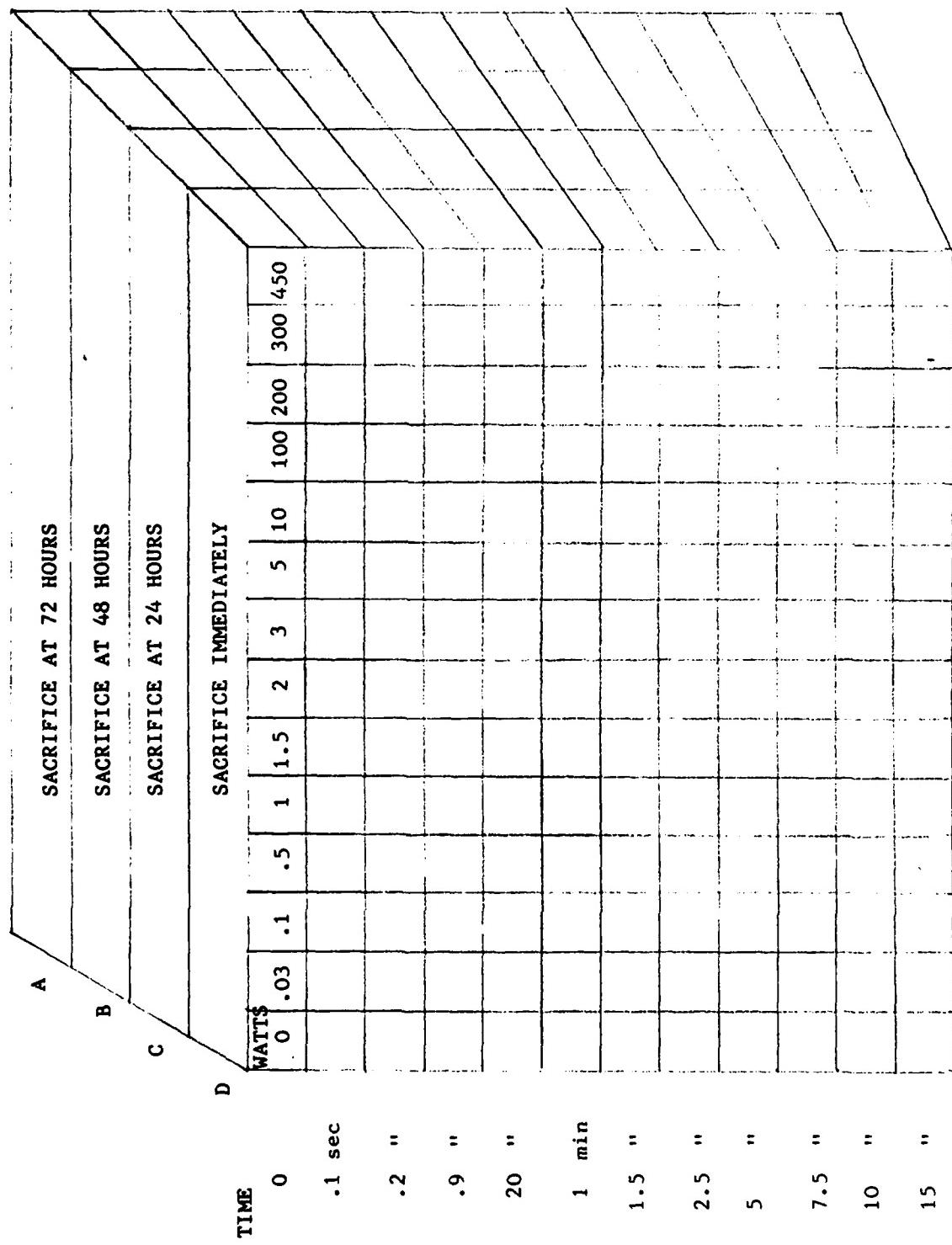
Table 1B

Sacrificed at 24 hr: CW

A — SACRIFICE AT 72 HOURS
B — SACRIFICE AT 48 HOURS
C — SACRIFICE AT 24 HOURS
D — SACRIFICE IMMEDIATELY

WATTS	0	.03	.1	.5	1	1.5	2	3	5	10	100	200	300	450

TIME

0
.1 sec
.2 "
.9 "
20 "
1 min
1.5 "
2.5 "
5 "
7.5 "
10 "
15 "

TABLE 2

#	Wattage	Time	Sacrifice	Air
1	0.5 W	10 sec	50 min	
	1.0	10		
	2.4	10		
	7.0	10		
2	0.5	10	50 min	+
	1.0	10		+
	2.0	10		+
	4.0	10		+
3	0.5	10	50 min	+
	1.0	10		+
	2.0	10		+
	5.8	10		+
4	0.5	10	50 min	+
	1.0	10		+
	2.0	10		+
	4.0	6		+
5	0.5	10		+
	1.0	10		+
	2.0	10		+
	3.7	10	50 min	+
6	0.5	10		+
	1.0	10		+
	2.0	10		+
	4.4	10	40 min	+
7	0.5	15	Immediately	+
8	1.0	15	44 hrs	+
9	--	--	--	Not Done
10	0.5	15	24 hrs	+
11	1.9	15	24 hrs	+
12	2.0	15	20 hrs	+
13	1.5	15	20 hrs	+
14	0	15	72 hrs	+
15	3.0	7.5	72 hrs	+
16	3.0	10.5	24 hrs	+

Table 2 Cont'd.

#	Wattage	Time	Sacrifice	Air
17	3.0 W	15 min	30 min	+
18	0	0	Immediately	+
19	2	15	48 hrs	+
20	2	15	Immediately	+
21	1	15	"	+
22	1	15	"	−
23	0.5	15	"	−
24	0.5	15	"	+
25	3.0	15	"	+
26	3.0	15	"	+
27	3.0	5	"	+
28	3.0	1.5	"	+
29	1.0	5	"	+
30	1.0	1.5	"	+
31	0.3	15	"	+
32	0.3	5	"	+
33	0.3	1.5	"	+
34	0.1	15	"	+
35	0	5	"	+
36	10	20 sec	"	+
37	100	0.9 sec	"	+
38	300	0.1 sec	"	+
39	300	0.2 sec	"	+
40	450	0.2 sec	"	+
41	0	1.5 min	"	+

Table 2 cont'd.

#	Wattage	Time	Sacrifice	Air
42	100	0.1 sec	Immediately	+
43	450	0.2 sec	"	+
44	0.1	5 min	"	+
45	0.1	1.5	"	+
46	0	15	"	−
47	0	15	"	+
48	--	--	--	−
49	.03	15	"	−
50	.03	15	"	−
51	.03	15	"	−
52	.03	15	"	−

TABLE 3

Rabbit #	Videoplan Assessed				Other Comment
	Left Control		Right Exposed		
1	--		--		Not assessed-handling too erratic. Both epithelial
2	X	X	X	X	and endothelial assessed only epithelial as yet
3	X	X	X	X	tabulated and in this report.
4	X	X	X	X	
5	X	X	X	X	One half of sample scratched and very dirty.
6	X	X	X	X	As above rabbit #2,3,4
7	X	X	X	X	" " " " " "
30					Not videoplan assessed as yet, pictures ready
31					Not videoplan assessed as yet, pictures ready
32					Not videoplan assessed as yet, pictures ready
36				X	Only right exposed pictures ready, not both assessed
43	X	X	X	X	
46					
47					Poor fixation specimen discarded
48					
49	X	X	X	X	
50	X	X	X	X	
52					Not videoplan assessed as yet, pictures ready
8	X	X			

TABLE 4

Percent of epithelial cells damaged as compared
to the total number of cells visualized at a
magnification of 500X at various predetermined
lines on the corneal surface

Rabbit #	Line Distance From Sclera	Left Eye Control	Damage Ave. %	Right Eye Exposed	Damage Ave. % ± SE	Comment
2	500 m	0%	.5%	10%, 0%	11%	Composite exposure.
		25%	8.3%	13%, 14%		
		0%		22%, 7%		
	1000 m	0%	0%	24%, 13%	13%	
		0%		21%, 2%		
				15%, 2%		
	2000 m	0%	1%	9%, 16%	11%	
		2%		10%, 6%		
				14%		
3	500 m	0%, 0%	1.8%	0%, 0%	0%	Composite exposure.
		0%, 9%		0%, 0%		
		2%, 0%		0%, 0%		
	1000 m	0%, 0%	0%	2%, 14%	4%	
		0%, 0%		0%, 3%		
		0%, 0%		2%, 5%		
	2000 m	5%, 29%	13.6%	40%, 17.6%	14%	
		30%, 0%		0%, 5%		
		7%, 11%		20%, 0%		
4	500 m	0%, 0%	0%	12.5%, 6%	9.25%	Composite exposure.
	1000 m	0%, 0%	0%	6.5%, 21.8%	14.2%	
	2000 m	0%, 0%	0%	20%, 12%	16%	

Table 4 Cont'd.

Rabbit #	Line Distance From Sclera	Left Eye Control	Damage Ave. %	Right Eye Exposed	Damage Ave. % ± SE	Comment
5	500 m	0%, 4% 0%	1.3%	18.4% 39.7%	29%	Composite exposure.
	1000 m	0%	0%	25%, 42.5%	34%	
	2000 m	0%	0%	26%, 22%	24%	
6	500 m	3.6%, 0%	1.8%	0%, 0%	0%	Composite exposure.
	1000 m	12%, 0%	6%	0%, 0%	0%	
	2000 m	0%, 2%	1%	0%, 0%	0%	
7	500 m	0%, 0%	0%	9%, 19.6%	14.3%	Composite exposure.
	1000 m	0%, 0%	0%	6%, 7%	6.5%	
	2000 m	1.7%, 0%	0.8%	12%, 21%	16.5%	
	500 m	0%, 0%	0%			Right eye not videoplan assessed yet, pictures ready.
	1000 m	0%, 0%	0%			
	2000 m	0%, 0%	0%			
36	500 m			0%, 0%	0%	Only one right specimen assessed 10W
	1000 m			0%, 9.4%	4.7%	
	2000 m			11%, 7%	9%	
43	500 m	0%, 7.3% 2%		0%, 100% 0%	33.3%	Note one area completely damaged while others not at all in right Exp. 450W
	1000 m	0%, 10.7% 3.6%	7.2%	0%, 100% 4%	34%	
	2000 m	0%, 0%, 0%	0%	0%, 100%, 0%	33.3%	
49	500 m	41%, 5%	23%	0%, 0%	0%	Note one left control poor quarter. Perhaps should be discarded? 0.03W
	1000 m	26%, 0%	13%	5%, 2%	3.5%	
	2000 m	41%, 0%	20.5%	4.4%, 4%	4.2%	

Table 4 Cont'd.

Rabbit #	Line Distance From Sclera	Left Eye Control	Damage Ave. %	Right Eye Exposed	Damage Ave. % ± SE	Comment
50	500	0%, 0%	0%	3%, 2%	2.5%	0.03W
	1000 m	0%, 0%	0%	1.5%, 0%	0.75%	
	2000 m	0%, 0%	0%	0%, 0%	0%	

TABLE 5: Summary

Rabbit #	Line Distance From Sclera	Left Eye Control	Damage Ave. %	Right Eye Exposed	Damage Ave. % $\pm$ SE	Comment
SEM	500 m	0%, 0%	0%	0%, 9%	4.5%	Handling(?) can
53	1000 m	25%, 5.5%	15.3%	0%, 5.5%	2.7%	cause small
SHAM	2000 m	0%, 0%	0%	0%, 0%	0%	damaged areas.
49	500 m	41%, 5%	23%	0%, 0%	0%	**GROUP A:** *One
	1000 m	26%, 0%	13%	5%, 2%	3.5%	of left quad-
	2000 m	41%*, 0%	20.5%	4.4%, 4%	4.2%	rants very poor
						cause unknown,
						(handling?).
						Results should
						be discarded?
						0.03W
50	500 m	0%, 0%	0%	3%, 2%	2.5%	Note left quad-
	1000 m	0%, 0%	0%	1.5%, 0%	0.75%	rant comparison
	2000 m	0%, 0%	0%	0%, 0%	0%	with above.
						0.03W
52	500 m	0%, 0%	0%	5%, 0%	2.5%	Most damage
	1000 m	0%, 0%	0%	0%, 23%	11.5%	seen here –
	2000 m	3.5%, 0%	1.7%	32%, 18%	25%	though immed-
						iately after
						experiment ap-
						peared un-
						touched. Cen-
						tral cornea
						most affected.
55	500 m	0%, 4%	2%	2.8%, 0%	1.4%	**GROUP B:** Did
	1000 m	1.4%, 2.6%	2%	5.6%, 9.8%	7.7%	excessive han-
	2000 m	5.6%, 3%	4.3%	11.6%, 14.8%	13.2%	dling of rab-
						bit caused more
						damage in con-
						trol animal?
57	500 m	4.8%, 6.2%	5.5%	10.9%, 18.2%	14.6%	
	1000 m	0%, 0%	0%	4.1%, 21.9%	13%	
	2000 m	0%, 0%	0%	4.2%, 23%	13.6%	
8	500 m	0%, 0%	0%	12.5%, 19.2%	15.8%	**GROUP C:** Pulsed
	1000 m	0%, 0%	0%	17.6%, 18.3%	18%	sample
	2000 m	0%, 0%	0%	12.2%, 13.3%	12.8%	1W

Summary Table Con'td.

Rabbit #	Line Distance From Sclera	Left Eye Control	Damage Ave. %	Right Eye Exposed	Damage Ave. %	Comment
59	500 m	14.7%		2.8%		Although only
	1000 m	0%		2.4%		one quadrant
	2000 m	0%		0%		viewed much
						less damage,
						seen in CW
						sample.
						1W
36	500 m	Not as yet		0%, 0%	0%	GROUP D: Left
	1000 m	videoplan		0%, 9.6%	4.8%	viewed on sem –
	2000 m	assessed		11%, 7.4%	9.2%	normal
43	500 m	7.3%, 20%	13.7%	100%, 0%	50%*	Complete total
	1000 m	10.7%, 3.6%	7.2%	100%, 4%	52%*	damage of one
	2000 m	0%, 0%	0%	100%, 0%	50%*	quadrant would
						suggest targe-
						geting of area
						other quadrant
						little or no
						damage.

FIGURE LEGENDS

Fig. 1.   Overview of rabbit in holder in front of antenna in exposure chamber.
           (a) View from front of antenna showing rabbit in carrier in front of
               round focusing antenna with eye positioned 2.5 Tem behind focus of
               antenna.
           (b) View from side of antenna showing typical view of right side of
               rabbit head as visible using video camera and thermographic camera.
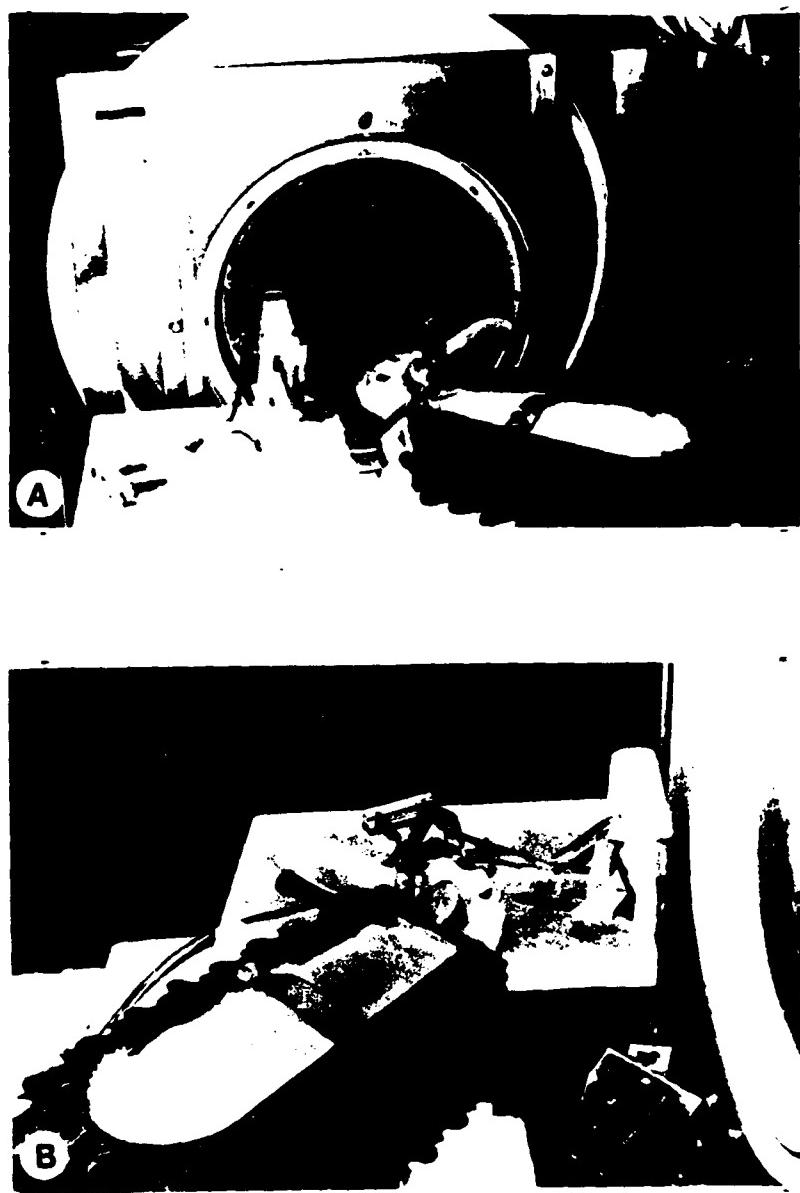
Figure 1

Fig. 2.   Typical images and thermal profiles of rabbit head in region of the eye, obtained using different representative techniques.

(a)   Photography.

(b)   Thermographic image using AGA Thermovision camera, range 20 degrees Celcius.

(c)   Map of temperature profiles of right side of rabbit head in the region of the eye. The temperature profiles were obtained by graphing the temperatures along lines drawn at constant vertical intervals across the map of the rabbit head temperatures.

(d)   One horizontal profile at line 28 of the two dimensional temperature map of the right side of the rabbit head, illustrating the flattened peaks found in the corneal region.

(e)   One vertical temperature profile at the division between the temporal (rear) third and the nasal (front) two-thirds of the eye (column 62).

(f)   A second vertical temperature profile at the division between the nasal (front) third and the temporal (rear) two-thirds of the eye (Column 75).
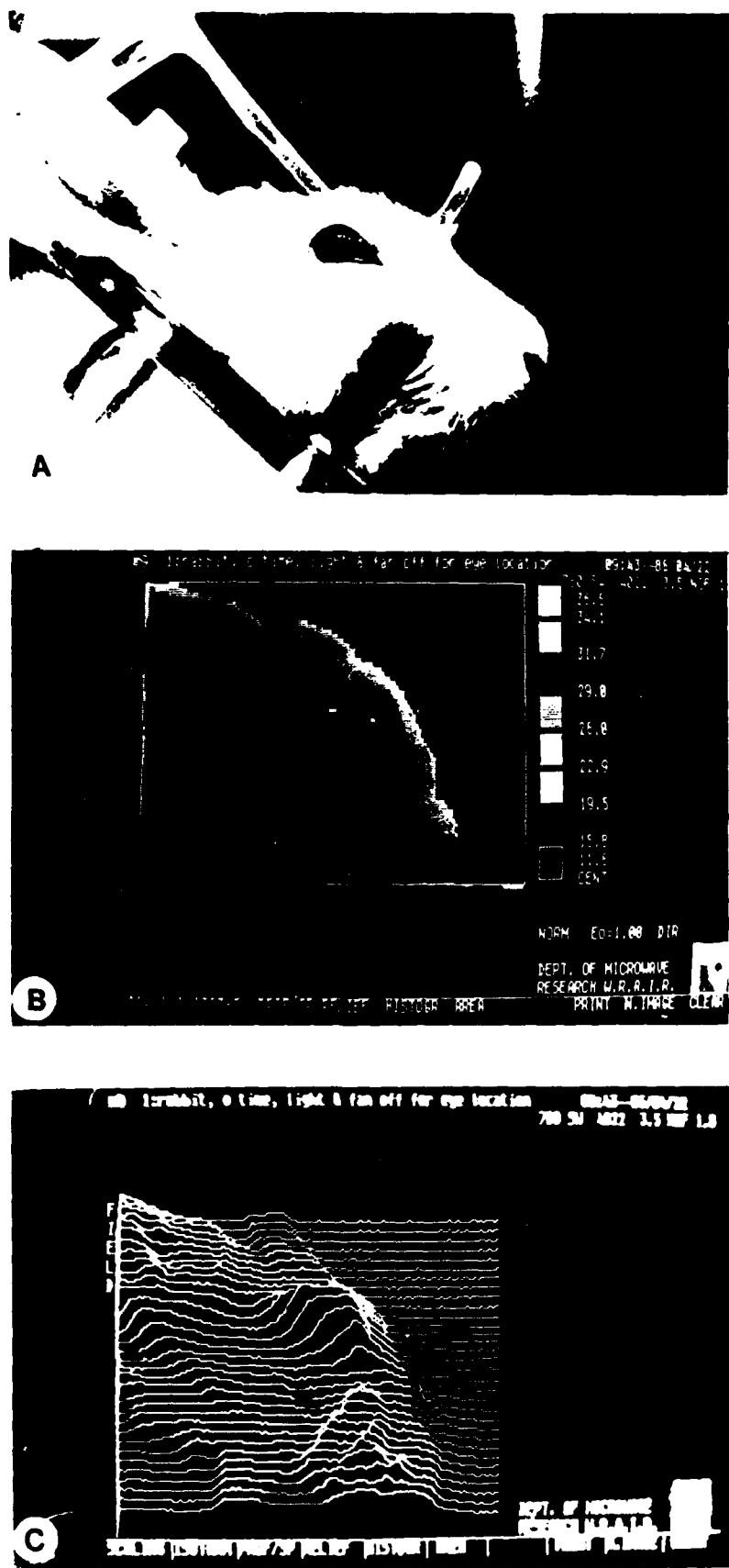
Figure 2
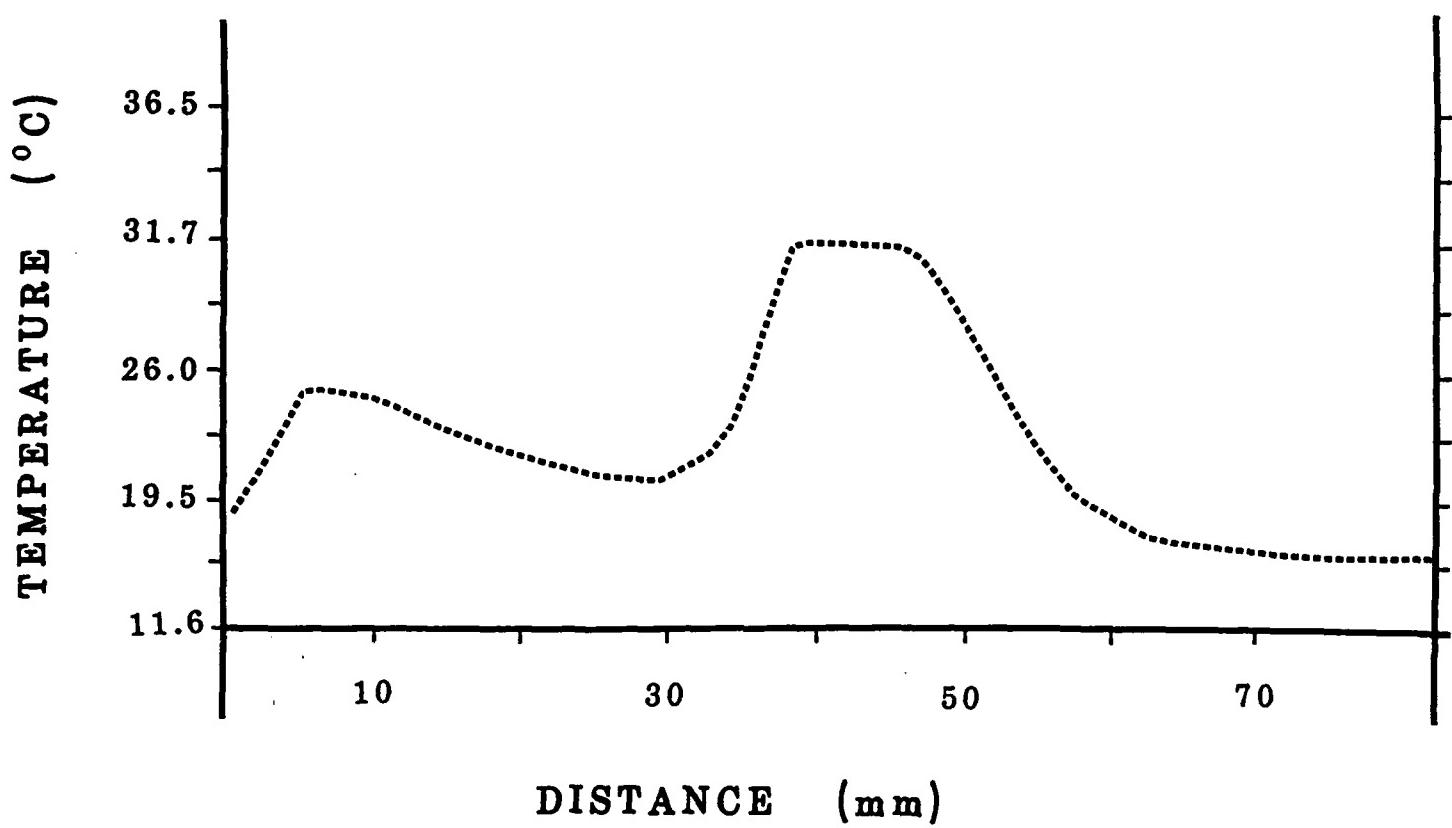
Figure 2d
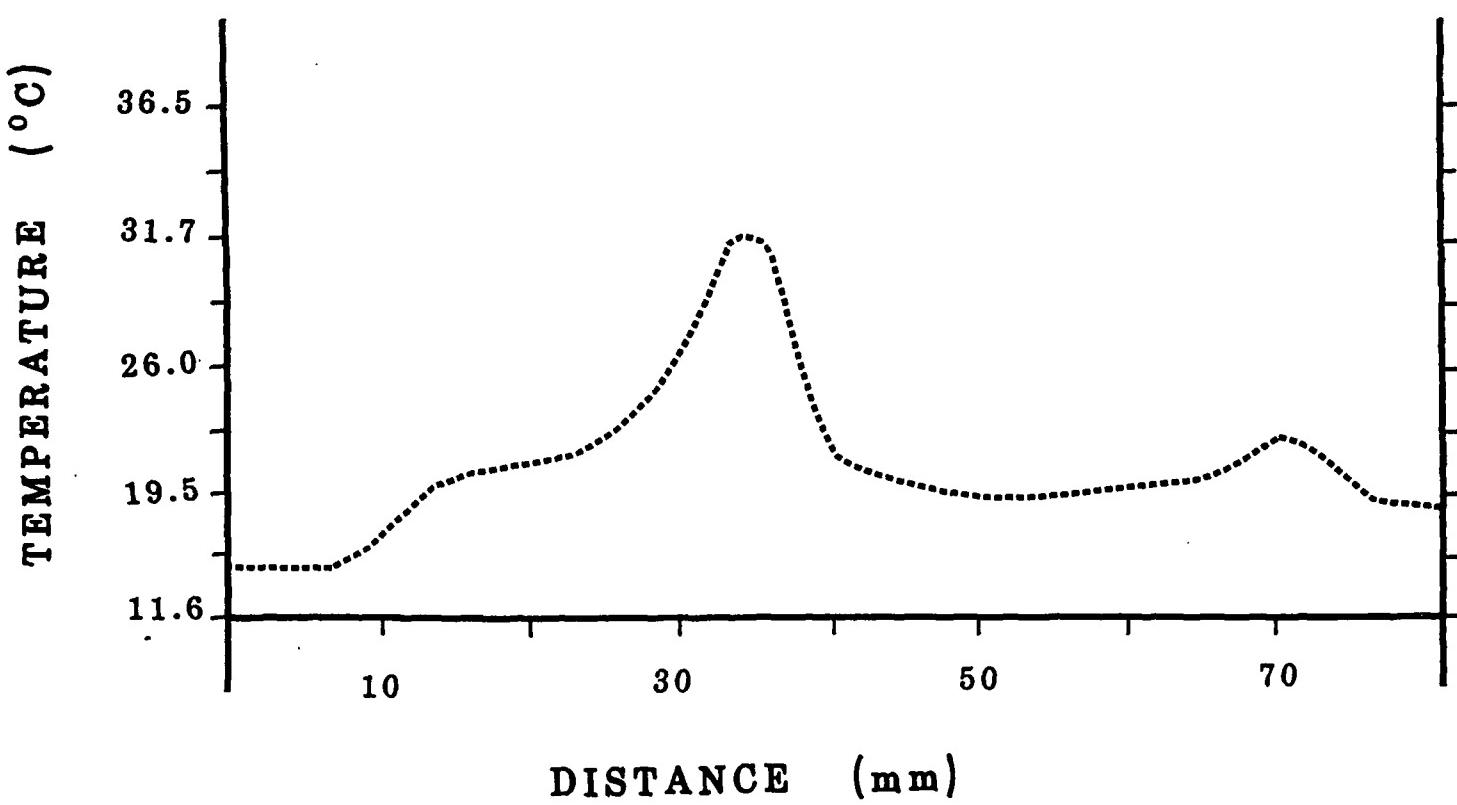
Figure 2e
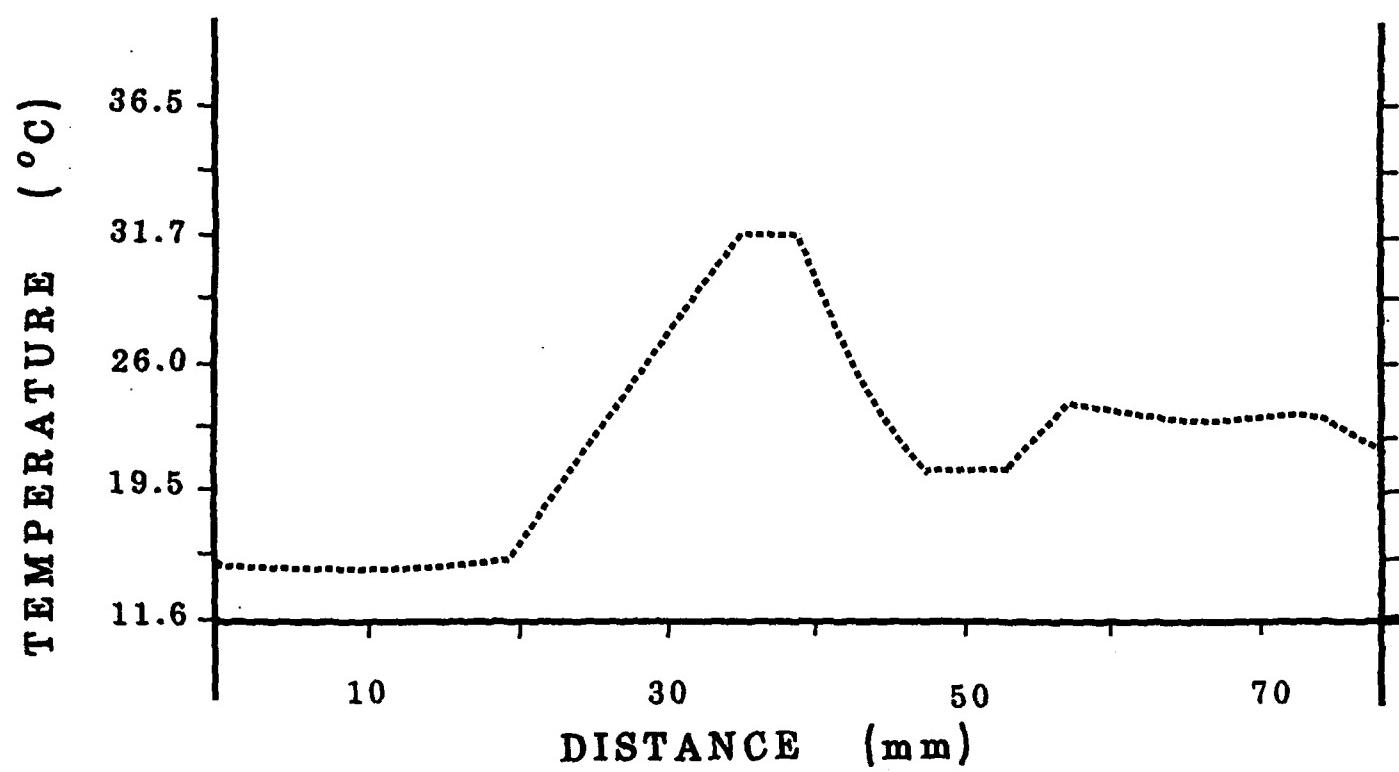
Figure 2f

Fig. 3.   Temperature images and graphs of profiles of the heating of the corneal
           phantom by the focused beam of millimeter waves from the antenna.
           (a) The temperature differences (before irradiation minus after
                irradiation) at each point of the thermographic image were plotted
                as a series of temperature profiles illustrating the spot heated by
                the millimeter wave beam at the beam focus.
           (b) The horizontal profile of temperature differences (final-initial)
                through the peak at the maximum temperature reached when the
                cucumber at the position of the beam focus was heated by the beam
                at an average power of 4 W using 8 pulses of 100 μsec and 20,000 kw
                peak power for 2 sec total.
           (c) The vertical profile through the peak point of maximum temperature
                difference (final - initial) reached when the cucumber was heated
                by the millimeter wave beam as in (a) at an average power of 4W.

5 cm
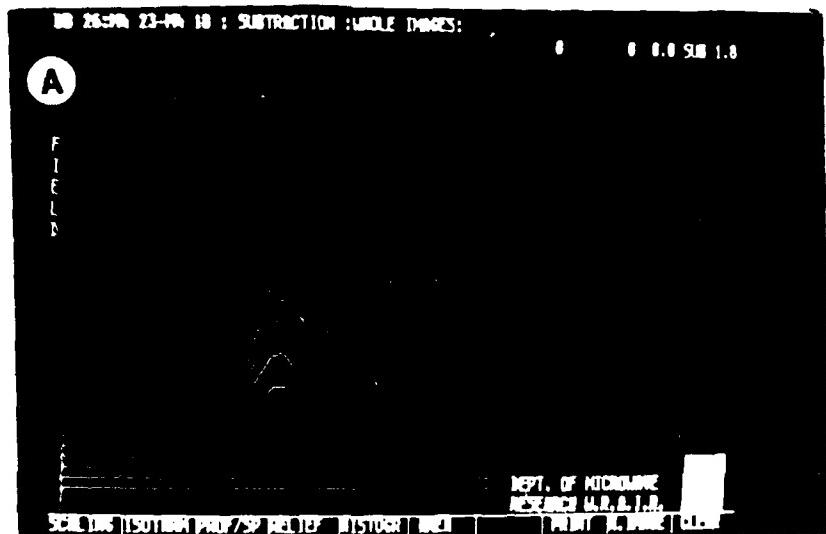
Figure 3
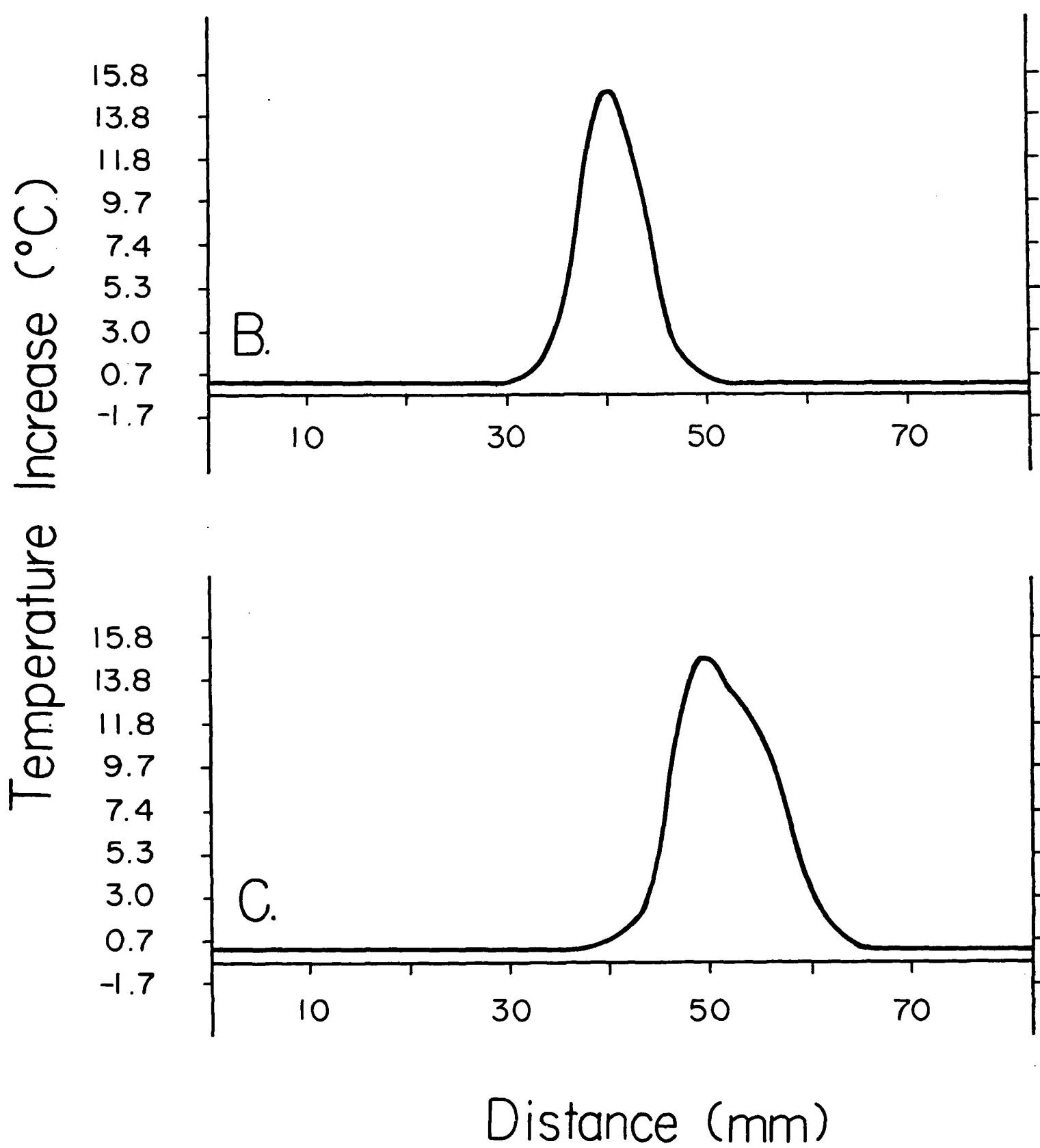
Fig. 3

Fig. 4.   Thermographic images and graphs of protiles of the temperature
            differences (final - initial) of the rabbit cornea heated by the focused
            beam of millimeter waves from the antenna.
            (a)   The temperature differences (before irradiation minus after
                  irradiation) at each point of the thermographic image were plotted
                  as a series of temperature profiles illustrating the spot heated by
                  the millimeter wave beam at a position 2.5 cm back of the beam
                  focus.
            (b)   The horizontal profile of temperature differences (final - initial)
                  through the peak at the maximum temperature reached when the rabbit
                  eye at the position 2.5 cm back of the beam focus was heated by the
                  beam at an average power of 3 W using pulses of 10 μsec and 22 kw
                  peak power for 1.5 min total.
            (c)   The vertical profile through the peak point of maximum temperature
                  difference (final-initial) reached when the rabbit eye was heated
                  by the millimeter wave beam as in (a) at an average power of 3 W.
            (d)   The vertical profile along a line 1/3 of the temporal-nasal
   distance across the rabbit eye following heating as in (c).

5 cm
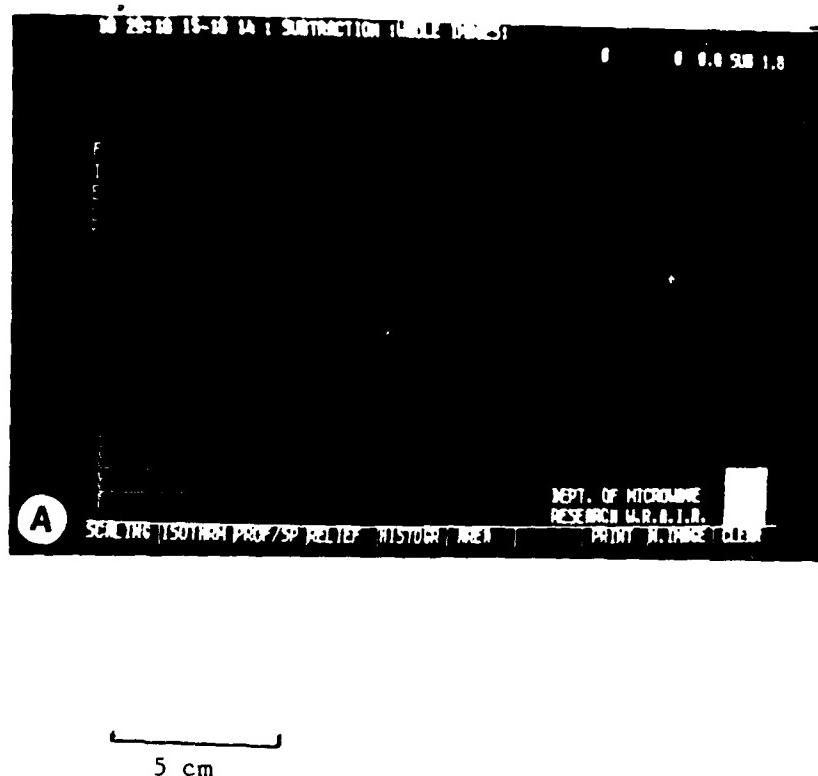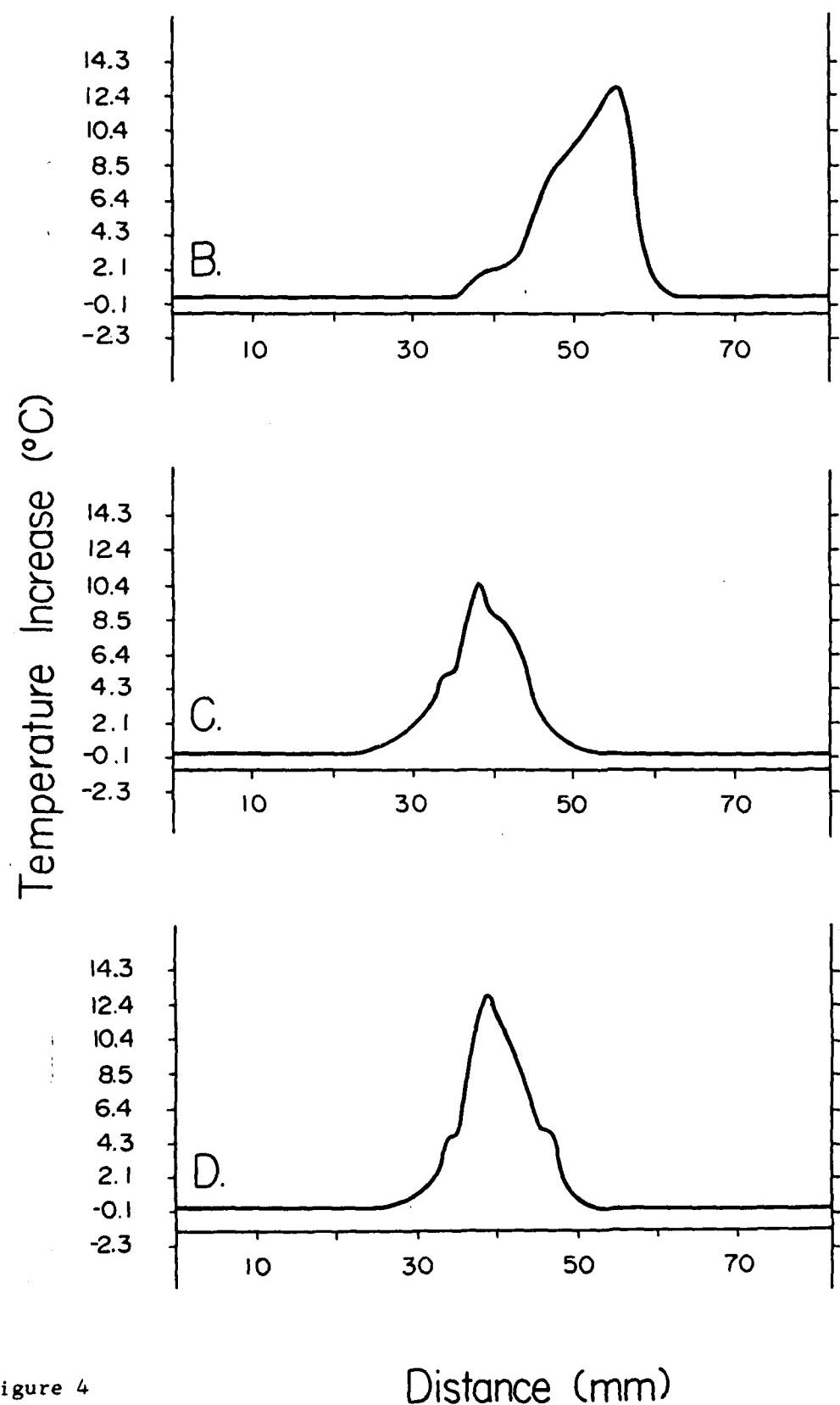
Figure 4

Figure 4

Fig. 5.   Cooling of the cornea by air flow. Change of temperature (cooling)
           during the first ten seconds of cooling air flow at different air flow
   rates.
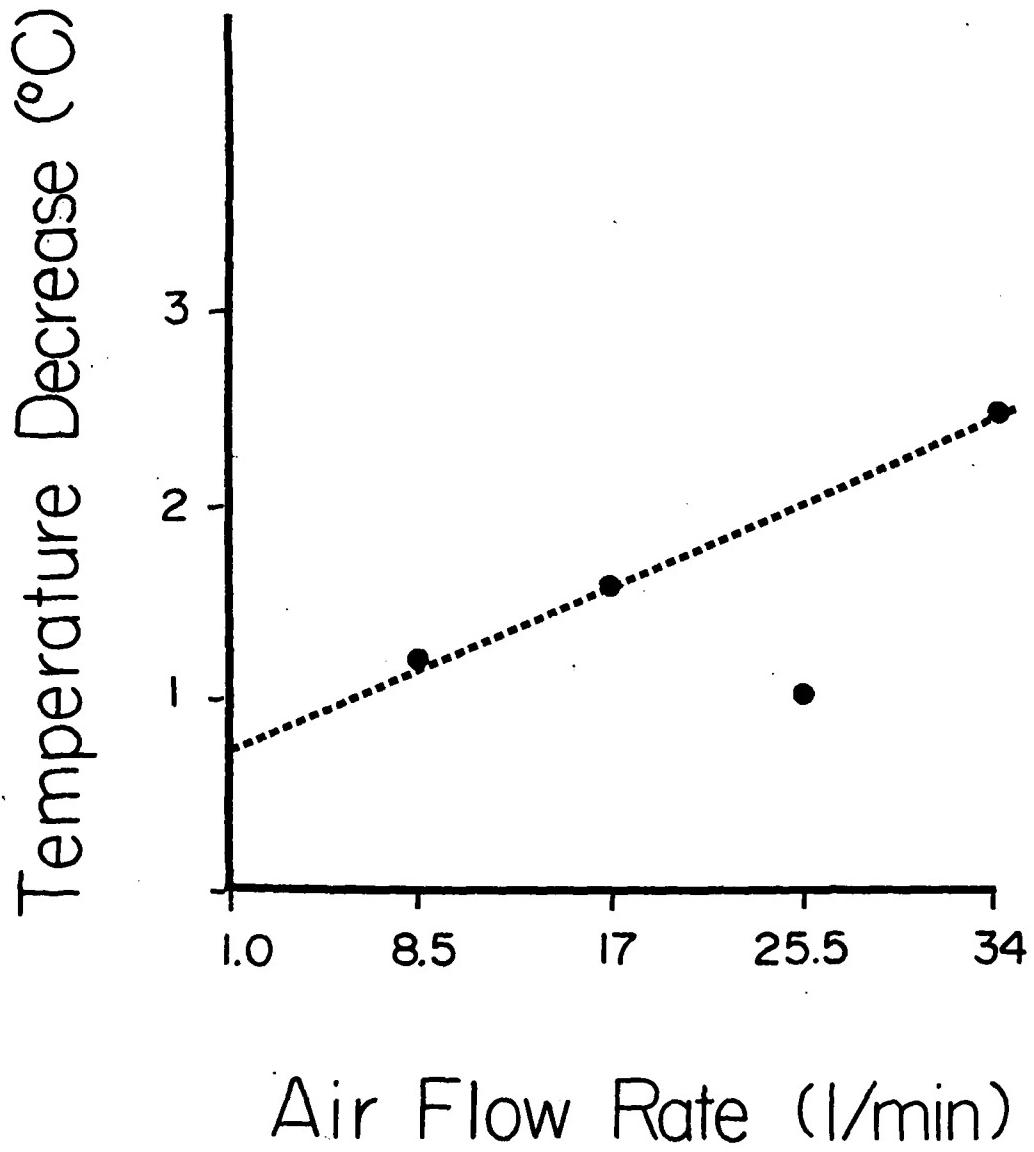
Figure 5

Fig. 6.   Temperature as a function of power applied using continuous wave
           millimeter waves of frequency 35 GHz, and dependence of temperature on
           rates of air pressure in cooling apparatus.
           (a) Dependence of temperature elevation at point of maximum temperature
                increase on power density at several different air flow rates.
           (b) Dependence of the temperature elevation on the air flow rate at
                constant power density. Note similar slopes of lines obtained for
                differenti powers, showing similar decrease in temperature with
                increases in air flow rate.

POWER (WATTS)

TEMPERATURE ELEVATION (^{0}C)
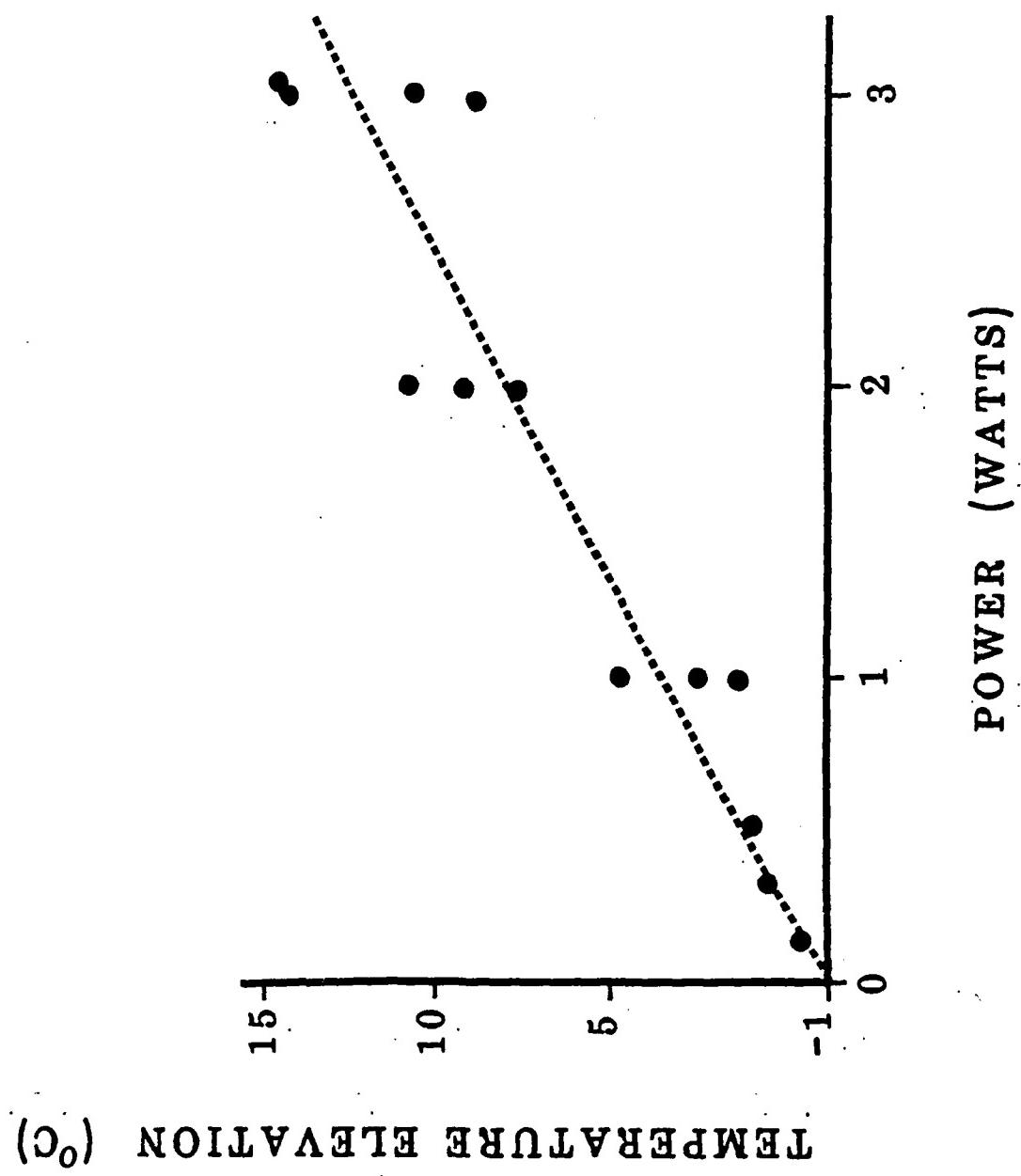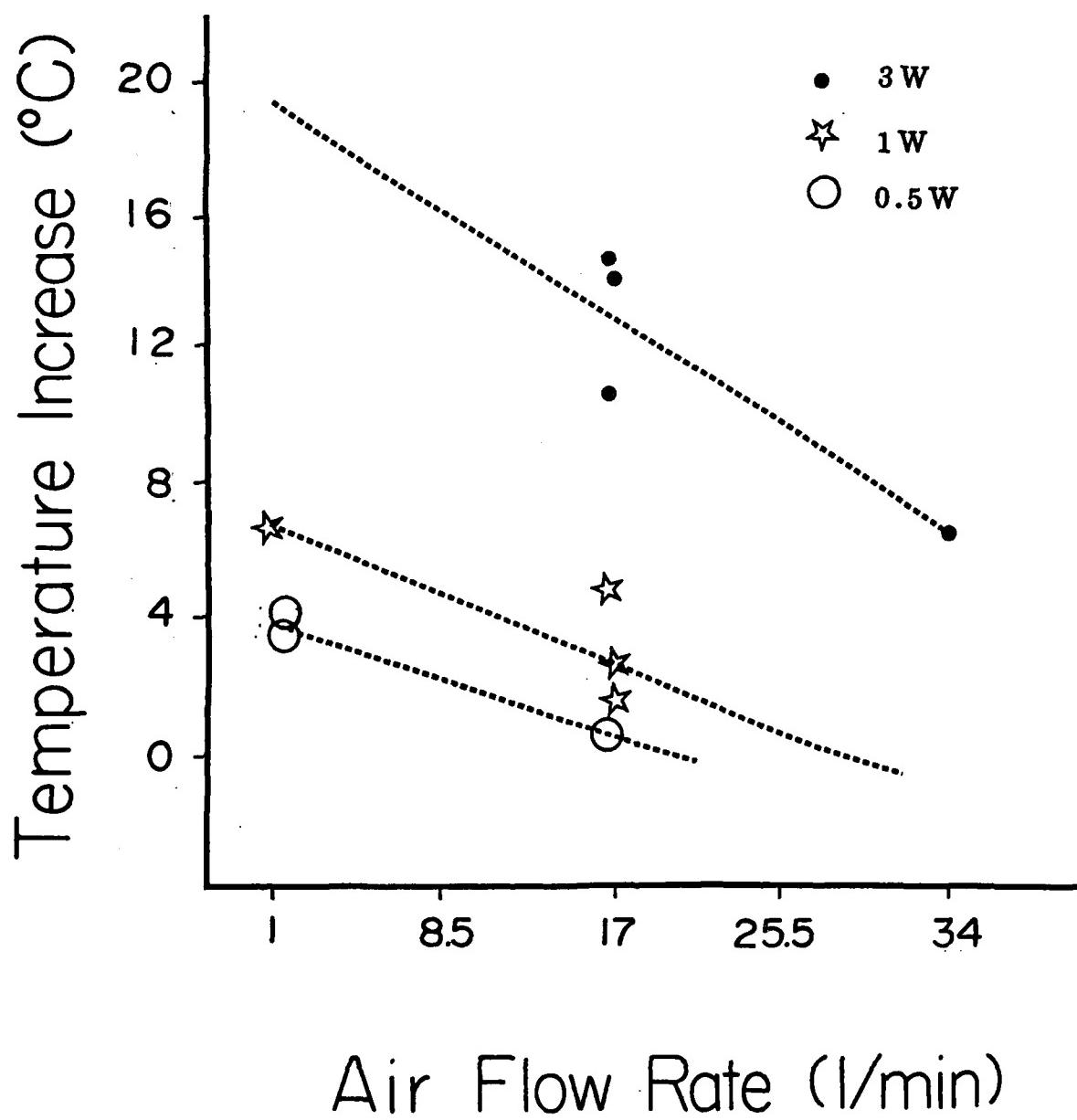
Figure 6a

Figure 6b

Fig. 7.   Dissection of cornea for SEM, TEM after irradiation in diagrammatic
   view.
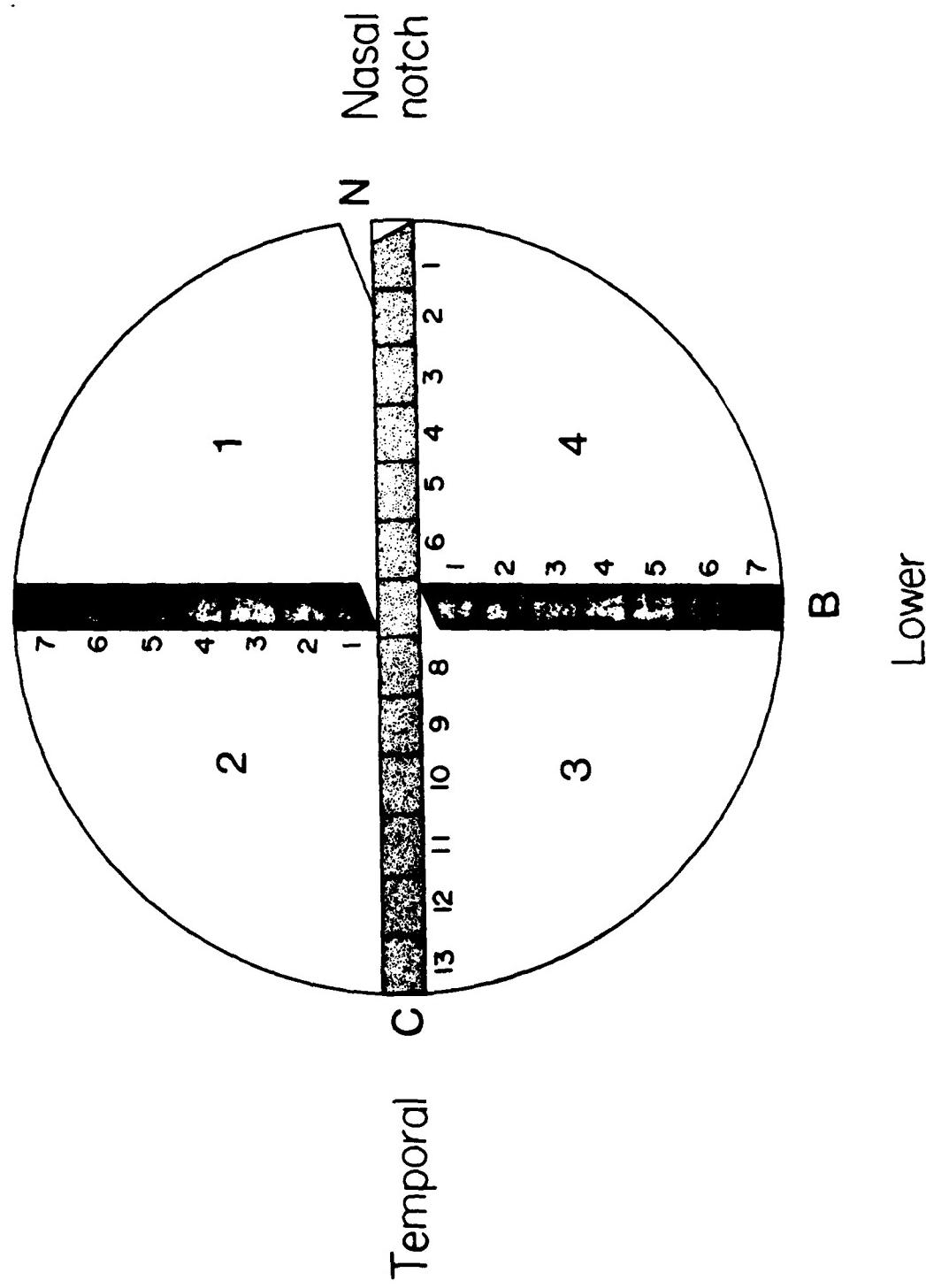
Figure 7

Fig. 8.   Scanning electron microscopy visual library of corneal damage for millimeter wave treated rabbits.

(a)   Control cornea from the left eye of a rabbit after millimeter wave exposure only on the right eye but illustrating light (1) and dark(2) cells. Note the crater like spots normally found on the surface of all cells even in normal animals. B, C, D show increasing degrees of damage to the corneal epithelial cells seen on the right eyes of three separate millimeter wave treated rabbits.

(b)   Type I – Epithelial damage in which a track-like array of single cell damage is observed. Cell damage apears due to both cell lifting and apparent heat coagulation (d). (Partial FSSD).

(c)   Type II – Area of epithelial damage is increased to include apparent groups of cells (5-8 cell total). Similar heat coagulated as in (b). (Partial FSSD).

(d)   Type III – In extensive area of epithelial damage and apparent heat coagulation is seen. Damage extends to cover standard screen area at standard 500x magnificantion (FSSD).
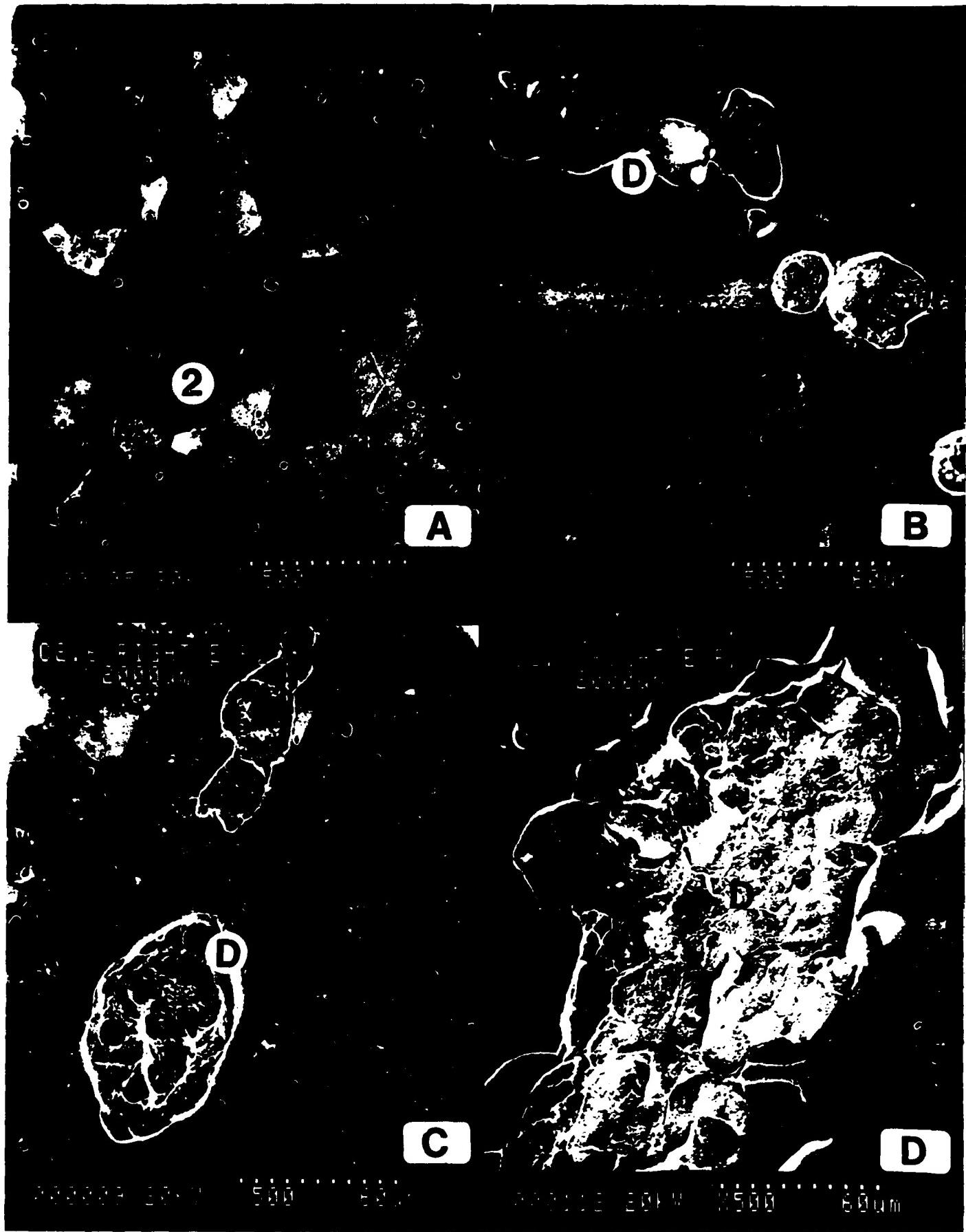
Figure 8

Fig. 9.   Cross section of millimeter wave treated rabbit corneas showing varying degrees of damage in the epithelial layers (EP), the apparently normal underlying stroma (5) and where appropriate areas of degeneration (D).

(a)   The outermost epithelial cells are lifting off singly as shown at

(b)   In addition to single cells lifting off (   ), there are groups of darkly staining degenerating outer epithelial cells (D).

(c)   Larger areas of cornea are involved and varying degrees of degeneration of the epithelial layers is seen (d). In some areas varying numbers of epithelial cell layers are lost, at places exposing the stroma (EX   ).

(d)   Occasionally the lower epithelial layers appear darkly staining indicating more degeneration (D) than in the outer epithelial layers (EP).
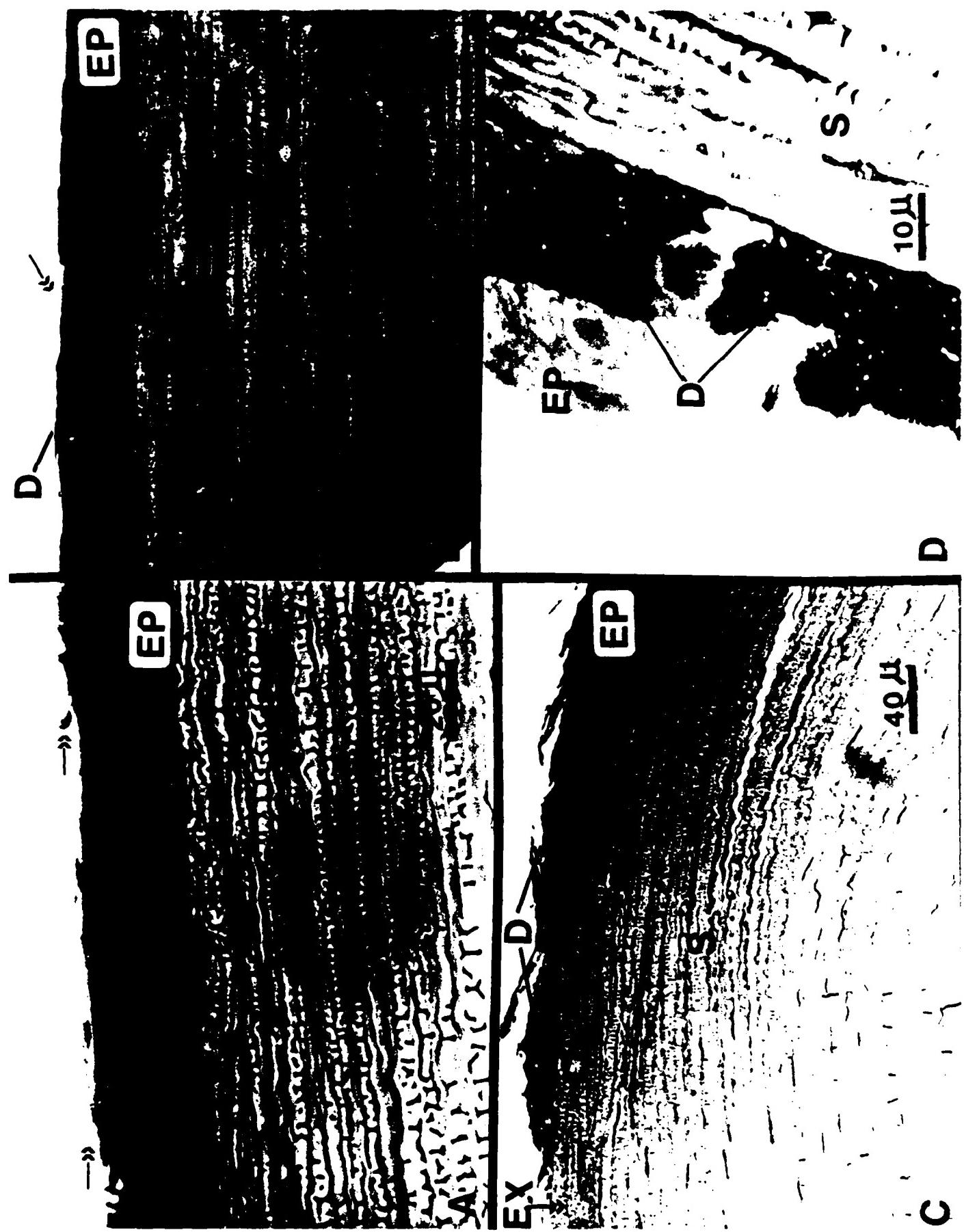
Figure 9

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